Accelerated Healing with a Mesh Autograft/Allodermal Composite Skin Graft Treated with Silver Nylon Dressings with and without Direct Current in Rats

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Purpose: **Evaluation of the healing and persistence of a meshed composite skin graft applied without immunosuppression.**

Methods: **The contraction of wounds grafted with 9:1 split-thickness autograft/1.5:1 allodermal mesh composite skin grafts (auto/allo MCSGs) was investigated. No immunosuppressive agent was applied. Male ACI rats and female Lewis rats reciprocally served as allodermis graft donors and recipients. Autograft/dermal autograft and allograft/dermal allograft MCSGs were the controls.**

Results: **At 3 months after grafting, when epithelized auto/allo MCSG wounds were measured by computerized morphometric analysis, the silver nylon (SN) dressing group displayed less contraction than the Vaseline (petroleum jelly) dressing group ($p < 0.003$), and direct current treatment (SNDC) was more effective than SN ($p < 0.005$). The histologic structures of the hair follicles appear to confine the rejection process to the allogeneic follicles of the graft. The focal nature of the rejection process and the relatively low antigenicity of the dermal matrix allowed the survival of the allodermis layer. Although direct current significantly enhanced MCSG healing, SN and SNDC were not the immunosuppressive agents that were confirmed.**

Conclusion: **This type of MCSG can heal without immunosuppressive treatment.**

Key Words: **Composite skin graft, Antigenicity, Immunosuppression, Silver nylon, Direct current**

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Prompt definitive coverage of the wounds of patients with extensive burns is commonly limited by a lack of available skin graft donor sites. In such patients, cutaneous allografts have been used for temporary coverage of excised burn wounds until reharvesting of the donor sites can be done. If left in place, allogeneic skin is rejected because epidermal cells, including Langerhans cells, contain the 1a surface membrane determinant, which is a highly active antigen to allogeneic recipients. Cultured keratinocytes and greatly expanded meshed autografts that are able to proliferate and cover the open wound beds have been used to avoid the allograft rejection reaction. However, those tissues are unable to protect the wounds from infection during the healing process and the formation and persistence of granulation tissue in the interstices of the widely meshed grafts results in uncertain engraftment, delayed healing, and consequent severe wound contraction.[1–9]

Various forms of a "deantigenized" dermis and a culture-derived dermal analog have been used to improve the engraftment and subsequent function of cultured keratinocytes and widely meshed cutaneous autografts. In the course of evaluating those "dermal" tissues, interest has focused on the development of a composite skin graft, consisting of allodermis and thin split-thickness autograft.[6–14] Allogeneic dermis has been used for the dermal component of composite skin grafts, because dermal collagen, chiefly type 1 collagen, is a rather poor immunogen in which the antigens are internal determinants that are exposed only after collagen denaturation. Dermal collagens, whether they are allogeneic or autogeneic, do not become denatured when transplanted if they receive early and sufficient blood supply and are protected from desiccation and infection. However, the dermal layer contains blood vessels and skin appendages such as hair follicles, and those cell components are highly antigenic.[15–21]

Early studies have shown that low-amperage direct current (DC) applied through a silver-impregnated nylon dressing (SN) controls infection, reduces edema formation, and improves the blood supply in partial thickness burns, split-thickness skin grafts, and skin graft donor sites, and then accelerates and improves their healing.[22–26]

The present study was conducted to evaluate the effect of low-amperage DC applied through a SN dressing on the epithelization and the reestablishment of the microcirculation in a composite, meshed allodermal, meshed thin split-thickness autograft. Special attention was focused on the effect of DC on the histologic changes that occur in the antigenically competent cells of the allogeneic dermis.

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MATERIALS AND METHODS

In conducting the research described in this report, the investigators adhered to the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and were in adherence with the *Guide for the Care and Use of Laboratory Animals,* NIH publication 80–23. The Animal Care and Use Committee of our institute approved experimental protocol and animal care.

Experimental Animals

A total of 465 white female Lewis rats, each weighing 200 ± 15 g, and 465 brown male ACI rats, each weighing 225 ± 25 g, were used as experimental animals. They were used reciprocally as allogeneic dermal graft donors and recipients. The main purpose of the experiments was to test the survival potential of an allogeneic dermal graft in a new mesh composite skin graft (MCSG) without pharmacologic immunosuppression. Histoincompatibility was the most important factor in selecting the two experimental rat strains. Accordingly, we selected the white Lewis and brown ACI rats as two highly incompatible inbred rat strains. We also selected different sexes to test the importance of the H-Y antigen. Because the H-Y antigen test is more sensitive in female animals, female white Lewis rats were the preferable recipients.[27] An additional 10 white female Lewis rats and 10 white male Hartley guinea pigs (400 ± 25 g) were used for the study of hair follicle vascularization.

Anesthesia

A balanced injectable anesthesia regimen effective in minimizing pain and distress without associated high mortality was developed and administered throughout the 2-hour operative procedure. Anesthesia was administered in two steps: 38 mg/kg of sodium pentobarbital was injected intraperitoneally as the basic anesthesia for preoperative preparation. Thereafter, 0.2 mL/kg of the anesthetic cocktail (ketamine HCl, 150 mg/1.5 mL; xylazine HCl, 30 mg/1.5 mL; and distilled water, 0.5 mL) was subcutaneously injected before surgery. During the operation, 16 mg/kg of sodium pentobarbital and 0.2 mL/kg of the anesthetic cocktail were alternately used for supplementation if necessary.

Experimental Groups

There were a total of nine experimental groups, as listed in Table 1. In each experimental group, half of the animals were Lewis rats and the other half were ACI rats.

Table 1 Experimental MCSG Groups

Group	Graft Type	Dressing	No. of Rats[b]
Control group[a]			
Allo/allo group			
1	Allo/allo	VG	70
2	Allo/allo	Wet SN	70
3	Allo/allo	40 μA anodal DC	70
Auto/auto group			
4	Auto/auto	VG	70
5	Auto/auto	Wet SN	70
6	Auto/auto	40 μA anodal DC	70
Treatment group			
Auto/allo group			
7	Auto/allo	VG	190
8	Auto/allo	Wet SN	190
9	Auto/allo	40 μA anodal DC	190

[a] Upper layer is always mesh 9:1 epidermal graft either autogeneic (auto) or allogeneic (allo). Underlying layer is always mesh 1.5:1 dermal graft.

[b] Half are female Lewis rats and half are male ACI rats.

Animal Models

After anesthesia was induced, the dorsal trunk of each rat was clipped and a depilatory cream (Nair, Carter-Wallace, Inc., Cranbury, NJ) was applied for 15 to 30 minutes, after which all hair was carefully washed off with warm tap water. Thereafter, the depilated dorsal skin of the operative field was under aseptic preparation. The method of graft harvesting from rodents has been previously reported.[25,26] A layer of split-thickness graft (0.012-inch thick) was first excised, then another layer of dermal graft (0.015-inch thick) was harvested from the same donor site of each rat. The residual dermis and panniculus carnosum of the wound were excised. The grafts and the open wounds each measured 4 × 6 cm. The split-thickness grafts were meshed in a 9:1 ratio and the dermal grafts in a 1.5:1 ratio by using a Zimmer Meshgraft II dermatome (Snyder Laboratories, Inc., Dover, OH). As indicated in Table 1, in the auto/allo experimental groups, the open wounds were first covered by a layer of 1.5:1 mesh dermal allograft, and then topped by a layer of 9:1 split-thickness autograft (0.012 in). A layer of Vaseline (petroleum jelly) dressing (VG), SN, or direct current treatment (SNDC) was then sutured over the MCSG wound.

The animal model with and without DC treatment has been previously reported.[23,25,26] When DC was applied, constant −40 μA DC was continuously applied for 5 days. All animals were individually caged and insulated from electrical conduction by using plastic cage stands. All cages were installed in an "animal intensive care unit chamber." Inside the chamber, temperature was kept between 78 and 80°C, with humidity of approximately 50% and appropriate ventilation. All animals were observed in the chamber for at least 48 hours after operation. All SN wound dressings were allowed to detach spontaneously after complete or nearly complete epithelization.

Evaluation of Wound Healing

Hair follicle vasculature distribution and graft circulatory reestablishment of both allogeneic and autogeneic layers of MCSGs were estimated from the presence of carbon particles in the blood vessels after arterial perfusion with 30 mL of Pelikan ink (Pelikan AG D300, Hannover, Germany) in rats (1–1.2 mL/10 g) and 50 mL of ink in guinea pigs (1–1.2

mL/10 g) at the speed of 38.2 mL/min by using an infusion-withdrawal pump (Harvard apparatus). Ink was perfused by means of a small polyethylene tube (PE-190: inner diameter, 1.19 mm; outer diameter, 1.70 mm) cannulated into the infrarenal aorta of the anesthetized rats and into the superior mesenteric artery of the anesthetized guinea pigs.

At gross examinations, wound epithelization, the presence of granulation tissue, hair growth and wound contraction were continuously observed and recorded for 3 months in 15 rats in the DC-treated auto/allo group, and in 10 rats in each of the other experimental groups. Photographs were taken of the animals at 14 (or 16, because of the time required for spontaneous detachment of the SN dressing), 21, 30, 60, and 90 days post grafting (PG). A centimeter marker was included inside the photo field. At 3 months PG, healed wound sizes were measured by computerized planimetric analysis (Optimas, BioScan, Inc., Edmonds, WA). Differences among group means (square centimeters and percentages) ± SEM were tested by using Bonferroni comparisons.

At microscopic examinations, 10 animals of each auto/allo experimental group were killed and samples of their grafts were examined microscopically at 2, 4, 7, 10, 14, 21, and 90 days PG. Additionally, five animals from each auto/allo group were killed and samples of their grafts examined microscopically at 3, 5, 12, 30, and 60 days PG. In allograft/dermal allograft (allo/allo) and autograft/dermal autograft (auto/auto) control groups, five animals of each group were killed and samples of their grafts examined microscopically at 4, 5, 7, 14, and 30 days PG, and in 10 animals samples of the grafts were examined microscopically at 90 days PG.

One square centimeter of the graft was excised from the center of the wounds of animals that had received auto/allo or auto/auto grafts. The excised graft specimens might include a thin layer of trapezius muscle and/or latissimus muscle, because the MCSG had been applied to a wound from which the panniculus carnosum, as well as epidermis and dermis, had been removed. The specimens excised from the healed wounds of allo/allo groups included both wound scar and adjacent healthy skin, because the wounds in these animals had formed linear scars by 90 days post burn. The tissue sections were stained with hematoxylin and eosin and Masson trichrome stains. Reestablishment of the microcirculation, reepithelization over the allodermal surfaces, necrosis of cells and tissues, degeneration and rejection of hair follicles, and dermal growth of normal collagen and/or fibrotic tissues were all assessed microscopically.

During the auto/allo MCSG healing process, the microscopic findings were analyzed very carefully, inasmuch as each MCSG had four different wound areas: (1) areas with both thin split-thickness autograft and allodermal grafts, (2) areas with only split-thickness autograft, (3) areas with only allodermal graft, and (4) areas of exposed wound bed. These four wound areas necessarily engendered recognizably different healing processes and results. Therefore, for analytical purposes, we decided that microscopic findings must have occurred in 50% or more of the examined wounds at a specific testing date to qualify as evidence of enhanced healing. However, specific phenomena that occurred occasionally but seemed to be important in demonstrating the mechanisms by which DC affected MCSG healing and immunologic responses were also reported.

We measured the dermis thickness and counted the hair follicle densities of the MCSG wounds at 3 months PG. These measurements were used for comparing the quality of dermal healing among the variously treated and untreated groups. To obtain these thickness measurements, a computerized planemetric program was used to measure the straight line distance from the surface of the muscle layer (the trapezius and/or latissimus muscles) to the epidermal-dermal junction. Ten measurements were obtained from each graft, and the mean for each examination time was calculated in 10 animals from each of the two auto/auto and three auto/allo groups. The method for measuring hair follicle density has been previously reported.[28] Data of microscopic measurements were statistically analyzed by using Bonferroni comparison.

RESULTS

Gross Examinations

In animal experiments, the results are strain-specific. In this report, we focus on the results obtained in the Lewis rat recipients. Because of the frequent disruption of healing caused by the self-destructive behavior of ACI rats, sampling was sporadic in those animals and the findings highly variable. Consequently, results obtained in the ACI rat groups are reported in summary form for reference only.

Allo/Allo MCSG Control Groups

The three allo/allo MCSG experimental groups with two allogeneic layers were the standard negative control groups for study of the auto/allo MCSG experimental groups. In all 10 allo/allo MCSGs treated with DC (control group 3), the allogeneic graft layers turned light pink, indicating revascularization by 4 days PG. No evidence of revascularization occurred in the other 20 allo/allo MCSGs not treated with DC. Thereafter, all 30 MCSGs of the three control groups desiccated, necrotized, and sloughed, leaving open inflamed wounds covered with granulation tissue by 16 to 24 days PG as a consequence of complete allograft rejection. Subsequently, these wounds healed in linear double Y-shaped scars with small foci of crust formation in 1 to 3 months PG. None of the wounds of the three allo/allo control groups showed evidence of significant wound epithelization. The rejection was not influenced by SN wound dressings treated with or without DC, which demonstrates that neither SN alone nor SN with DC application influenced the immunologic response to the allo/allo MCSG.

Auto/Auto MCSG Positive Control Groups

The following three groups were positive controls.

Table 2 Wound Sizes of Experimental MCSG Wounds of Recipient Lewis Rats at 3 Months Post Grafting

Group	VG	SN	SNDC
Auto/allo MCSG			
Size (cm^2) ± SEM[a]	7.89 ± 0.5	10.37 ± 0.7	13.56 ± 0.7
% of original area[b]	32.88	43.21	56.49
Auto/auto MCSG			
Size (cm^2) ± SEM	15.04 ± 1.6	14.39 ± 0.8	15.33 ± 0.9
% of original area[b]	62.65	59.93	63.89

[a] Auto/allo: SNDC vs. SN, $p < 0.005$. SNDC (or SN) vs. VG, $p < 0.003$.
[b] Original excision wound = 24 cm^2.

VG Group: At 24 days PG, in all but one rat of this group, MCSGs were not completely epithelized. At 3 months PG, all 10 MCSGs with VG dressings had largely healed with moderate wound contraction (37.35%) and sparse hair growth. However, 5 of 10 animals still had small open areas of granulation tissue.

SN Group: Complete epithelization occurred in the 10 of 10 experimental MCSG wounds at 14 days PG. No areas of granulation tissue developed in the following 3 months. At 3 months PG, the MCSGs had healed with moderate wound contraction (40.07%) and sparse hair growth.

SNDC Group: At 12 days PG, MCSGs were completely epithelized in 10 of 10 animals. No areas of granulation tissue developed in the following 3 months. At 3 months PG, MCSGs had healed with moderate wound contraction (36.11%) and moderate hair growth at the original meshed autograft sites.

Wound size measurements are listed in Table 2 and showed no significant differences among these three groups.

Auto/Allo MCSG Treatment Groups

VG Group: At 14 days PG, in 10 of 10 MCSG wounds, portions of allogeneic mesh dermal grafts not covered by mesh autografts were desiccated and necrotic. Such desiccation and infected necrosis of the mesh dermal allografts were obvious, as shown in Figure 2*A* at 14 days PG. However, portions of these MCSGs having mesh autograft coverage were well protected. Open wounds appeared interstitially among the mesh autografts after slough of the necrotic mesh dermal grafts. MCSG wounds needed more than 3 months for complete epithelization as shown in Figure 1 at 3 months PG. The epithelized areas of these wounds healed with severe contracture to 32.88% of original wound area (Table 2).

SN Group: Ten of 10 MCSG wounds achieved nearly complete epithelization at 14 to 16 days PG. A very thin layer of detached stratum corneum adhered to the SN dressings after spontaneous separation from the (epithelized) MCSG at 16 days PG. This finding indicates that the meshed dermal allograft layer was intact and had healed on the wound bed without rejection. Only 2 of these 10 animals had one to two small sites of granulation tissue present, which reepithelized rapidly in 24 to 60 days PG. At 3 months PG, these 10 MCSG wounds were completely epithelized, contracted to 43.21% (mean) of original wound area, and grew without hair but good skin texture.

Fig. 1. *Auto/Allo MCSG of the VG group. At 3 months PG, most of this wound had epithelized without hair growth. One area of exposed granulation tissue remains.*

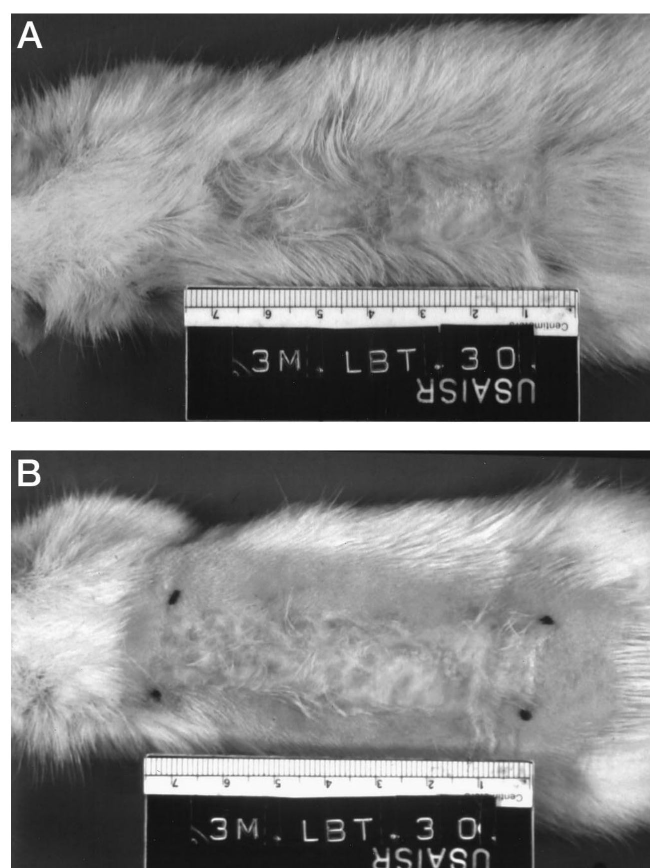

Fig. 2. *Auto/allo MCSG of the SNDC group. (A) At 3 months PG, MCSG had epithelized a mildly contracted wound (mean = 56.49% of original wound size) with sparse hair growth. (B) Healed wound after hair clipping.*

Table 3 **Wound Sizes of Experimental MCSG Wounds of Recipient ACI Rats at 3 Months Post Grafting**

Group	VG	SN	SNDC
Auto/allo MCSS			
Size (cm^2) ± SEM[a]	4.35 ± 0.7	9.83 ± 0.7	9.73 ± 0.7
% of original area[b]	18.14	40.95	40.54
Auto/auto MCSG			
Size (cm^2) ± SEM	4.97 ± 0.6	8.01 ± 0.7	8.10 ± 0.6
% of original area[b]	20.71	33.37	33.75

[a] Auto/allo: SNDC (or SN) vs. VG, $p < 0.003$.
[b] Original excision wound = 24 cm^2.

SNDC Group: With DC application, the MCSGs showed more successful healing. These 15 MCSG wounds completely epithelized with stratum corneum formation in 12 to 14 days after grafting. As seen in the SN animals, a very thin layer of detached stratum corneum adhered to the SN when it spontaneously detached from the epithelized wound. No areas of granulation tissue appeared in the following 3 months. At 3 months PG, all 15 MCSGs were healed with complete epithelization, contracture to 56.49% of the original wound area, with sparse hair growth at the original meshed autograft site, as shown in Figure 2, *A* and *B*.

In the female Lewis recipient rat group, the mean and SEM for wound size of these three auto/allo groups are presented in Table 2. Differences among group means as tested with Bonferroni comparisons were highly significant ($p < 0.003$). MCSG wounds contracted to 32.88%, 43.21%, and 56.49% of the original wound sizes in the VG, SN, and SNDC groups, respectively. It should be noted that the size of the thin split-thickness autograft used in this MCSG before being meshed at a 9:1 ratio equaled only to 11.11% of the original wound size (100%/9).

In the other experimental groups, when ACI rats were used as recipients, graft take was influenced by self-inflicted injuries. Open wounds frequently occurred in most of the experimental ACI rats during the 3-month observation period. Wound healing could not be estimated reliably for comparison among these experimental groups. However, data from the auto/auto control groups and the auto/allo treatment groups (Table 3) suggest that auto/allo MCSG treated with SN dressings with or without DC application enhances graft take and reduces wound contraction.

Microscopic Examinations

Blood Vessel Distribution in the Normal Hair Follicles

Histologic structures appear to influence the rejection process of allogeneic hair follicles in auto/allo MCSG healing. Normal hair follicles of rats and guinea pigs are sheathed within two layers of tight connective tissue.[28] In both rats and guinea pigs, the blood supply of the hair follicle consists of a single arteriole penetrating the sheath at the apex of the papilla (Fig. 3, *A* and *C*). Before entering the papilla, the arteriole divides into internal and external capillary plexuses (Fig. 3, *A* and *C*).

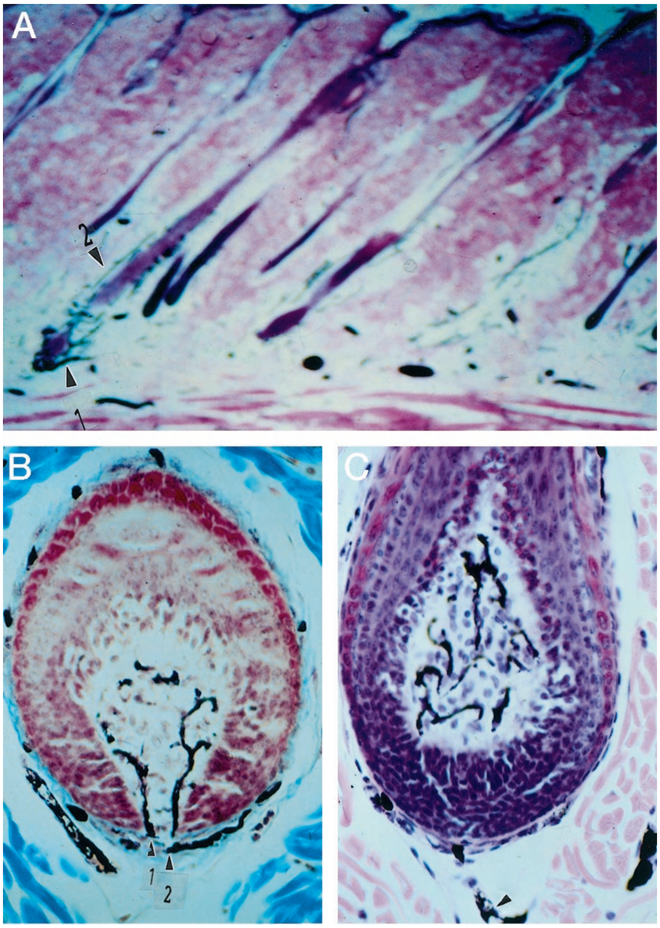

Fig. 3. *Vascular distribution of the normal hair follicles. (*A,B*) A single arteriole enters the rat (*A*; original magnification, ×25) and guinea pig (*B*; original magnification, ×120) hair follicles (arrowheads) (hematoxylin and eosin stain). No other arterioles and capillaries pass through the sheath. Before entering the papilla, the arteriole divides into two capillaries: (*A,C*) the internal capillary plexus entering the papilla (arrowhead 1) and the external capillary plexus surrounding the hair follicle inside the sheath (arrowhead 2) of the hair follicles of rat (*A*) and guinea pig (*C*; original magnification, ×120; Masson trichrome stain).*

MCSG Healing

Because the histologic healing process of the auto/auto MCSG is not obviously different from split-thickness autograft healing on an excision, which includes a thin layer of dermis as we have previously reported[28] and because the allo/allo MCSGs were completely rejected in all groups, in this study we focus on the results obtained from the three auto/allo MCSG groups by using Lewis rats as recipients. Five characteristic microscopic findings of MCSG healing are described below.

1. Graft Revascularization: In DC-treated wounds, vascular circulation of the MCSGs, in either both layers of auto/allo and allo/allo grafts or the single layer of allodermal grafts without meshed split-thickness autograft coverage, was reestablished in the 4th day PG as shown in these auto/allo (Fig. 4) and allo/allo MCSG (Fig. 5). Significant dilation and

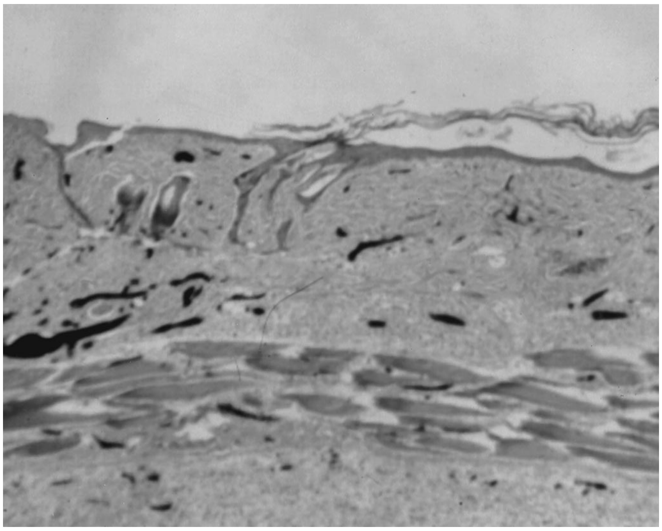

Fig. 4. *An auto/allo MCSG treated with DC at 4 days PG after ink perfusion. Hematoxylin and eosin stain; original magnification, ×25. Significant dilation and increase in the number of ink-filled vessels are noted in the allodermal layer.*

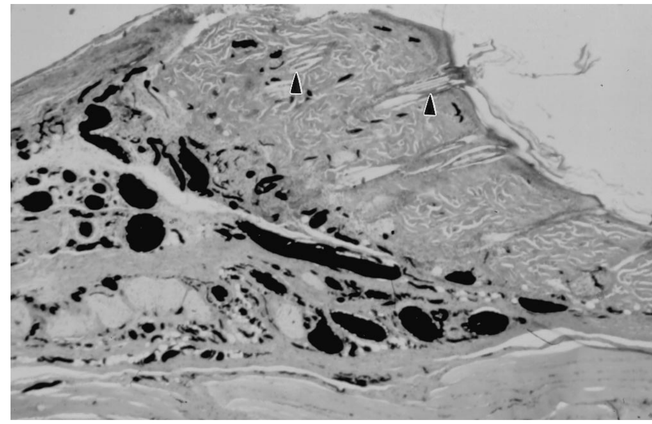

Fig. 5. *An allo/allo MCSG treated with DC at 4 days PG after ink perfusion. Hematoxylin and eosin stain; original magnification, ×25. Significant dilation and increase in the number of ink-filled vessels are obvious in both allogeneic split-thickness graft and dermal graft.*

increase in the number of ink-filled vessels in the allodermal layers of auto/allo and allo/allo MCSGs (Figs. 4 and 5) are apparent compared with the vasculature of healthy skin. To confirm this finding, measurements were obtained of transversely cut vessels of the allodermal layer containing ink in both auto/allo and allo/allo MCSGs at 4 days PG, as presented in Tables 4 and 5. The mean diameter ($n = 10$) of the largest transversely cut vessels of the SNDC groups were twice that of the healthy skin and VG and SN groups. The number of vessels in the SNDC groups was four times that of healthy skin and VG groups, possibly as a result of early recruitment of vessels in the dermal allograft, as a consequence of the increased blood flow induced by DC. Although graft revascularization did occur under DC treatment in the allo/allo MCSG at 4 days PG (Fig. 5), thrombosis did occur at 5 days PG and was followed by total rejection. In contrast, the allodermis layer of the auto/allo MCSG, under DC treatment, exhibited a rich circulation and progressive reepithelization on its surface at 5 days PG.

Without DC application, 5 to 7 days were required for graft revascularization in the auto/auto and auto/allo MCSGs of the SN groups, and revascularization did not occur at all in the allo/allo MCSGs. No vessel dilation was observed in any MCSG groups not receiving SN or SNDC treatment.

2. Epithelium Proliferation and Reepithelization: DC application accelerated epithelial proliferation, either autogeneic or allogeneic. At 5 days PG, most of the open wound areas and allodermal surfaces were covered by a layer of new autogeneic epidermis without mature stratum corneum in all auto/allo MCSGs. This healing required more than 7 days in the SN and VG groups. Even in the allo/allo MCSG group, DC treatment enhanced allogeneic epithelial proliferation

Table 4 **Diameter of the Largest Transversely Cut Allodermal Vessels Containing Ink (Mean ± SEM) at 4 Days Post Grafting**

Group ($n = 10$)	Unburned Skin	Allo/allo MCSG SNDC[a]	Auto/allo MCSG		
			VG[b]	SN	SNDC[a]
Diameter (μm)	58.6 ± 5.7	114.25 ± 7.9	52.9 ± 4.7	72.0 ± 4.4	109.2 ± 8.5

[a] SNDC (auto/allo or allo/allo) vs. SN, $p < 0.002$.
[b] VG vs. SN, $p < 0.001$; VG vs. SNDC, $p < 0.001$.

Table 5 **Number of Ink-Containing Vessels in Allodermal Layer of MCSG and in Unburned Skin (Mean ± SEM) at 4 Days Post Grafting**

	Unburned Skin[a] (deep 2/5th dermis)	Allo/allo MCSG[a] SNDC[b]	Auto/allo MCSG[a]		
			VG	SN	SNDC[b]
No. of vessels	20.9±3.3	105.7±4.7	27.1±4.2	52.9±4.1	117.5±5.2

[a] Average in two low-power fields in one histological section for each of 10 animals.
[b] SNDC vs. VG, $p < 0.001$; SNDC vs. SN, $p < 0.001$; SNDC vs. unburned skin, $p < 0.001$.

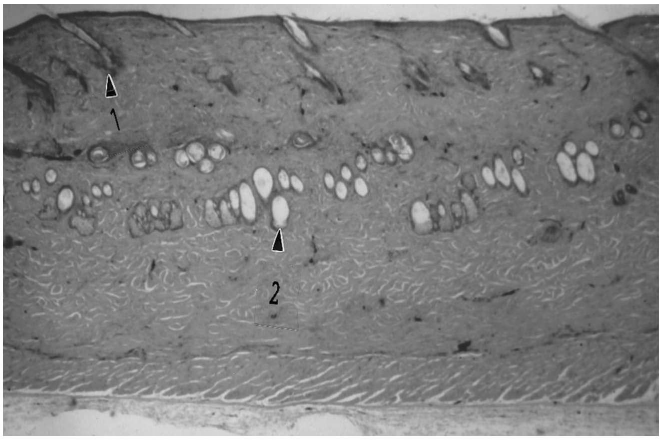

Fig. 6. *An auto/allo MCSG treated with DC at 7 days PG after ink perfusion. Hematoxylin and eosin stain; original magnification, ×25. The ink filled vessels were not dilated. Degeneration of the hair follicles in the superficial autograft layer is obvious (*arrowhead 1*). Cystic degeneration caused by local rejection (*arrowhead 2*) of the allogeneic hair follicles in the underlying allodermal layer is apparent.*

over focal small areas of the excised wound bed and the allodermal surface at 4th and 5th days PG. However, heavy white cell infiltration indicated that host intolerance to the allografts was already evident. These apparently allogeneic epidermal cells subsequently underwent necrosis and sloughed during the allograft rejection process on and after 7 days PG. Epithelial proliferation did not occur in either VG or SN allo/allo MCSG groups.

3. Survival of the Autogeneic Hair Follicles and "Local Rejection" of the Allogeneic Hair Follicles: Degeneration of the autogeneic hair follicles and rejection of the allogeneic hair follicles were not obvious at 3 days PG but began at 4 days PG. In the meshed split-thickness autograft layers, degeneration of the autogeneic hair follicles, either undamaged or partially excised, began at 4 days PG and was very obvious at 7 days PG, as shown in Figure 6 (*arrowhead 1*). After 7 days PG, such hair follicles progressively degenerated and subsequently disappeared in the VG and SN groups. In the SNDC group, the degenerative process often ceased short of total destruction with conversion to regeneration and growth of the follicles into the underlying allodermis layer (Fig. 7, *arrowhead*). Normal hair follicles developed as early as 14 days PG (Fig. 7, *arrowhead*) and were typically evident at 3 months PG. At 3 months PG, hair follicles were commonly found only in the original autograft portion of auto/allo MCSGs treated with DC.

In the allodermal layers, a few small cysts containing hair shaft remnants and debris from cellular rejection inside the hair follicle sheaths were found in SNDC group as early as 4 days PG. At 7 days PG, such cysts were apparent in nearly all allogeneic hair follicles (Fig. 6). As the dermis thickened, the cysts typically expanded, gradually moved superficially (Fig. 8), and then eroded into the autoepidermis and expelled out from

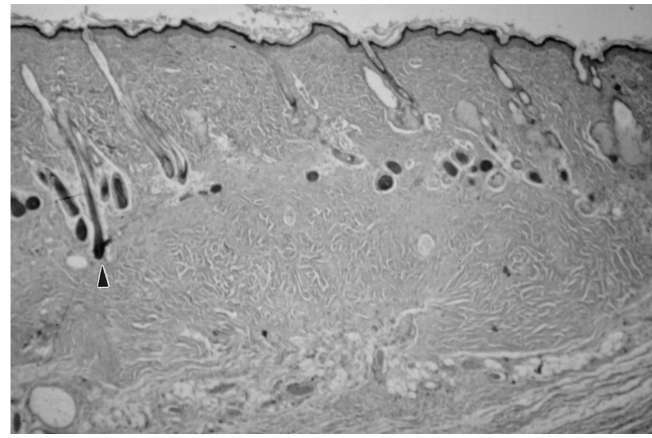

Fig. 7. *An auto/allo MCSG treated with DC at 14 days PG. Hematoxylin and eosin stain; original magnification, ×25. A hair follicle is growing from the autograft layer into the underlying allodermal layer (*arrowhead*).*

the MCSG (Fig. 9). During the process of rejection of the allogeneic hair follicles, neither inflammation nor other signs of tissue damage were evident in the adjacent dermal matrix. We consider this type of rejection to be a "local rejection." Such "local rejection" also occurred in VG and SN auto/allo MCSG animals but began at or later than 7 days PG. The histologic findings in the SNDC group indicate that DC accelerated the "local rejection" 3 days before that in the SN and VG groups. This finding seems to be related to the earlier reestablishment of the MCSG microcirculation. Table 6 shows the number of animals with cyst formation in variably treated auto/allo MCSG at six different times from 4 to 90 days PG. The significance of the observation is that all the allogeneic hair follicles are deleted by the "local rejection," regardless of the wound dressing used. The data in Table 6 identify that the local rejection begins at 4 days PG in the SNDC group and 7 days in the SN and VG groups and indicate that the process can continue for more than 3 months.

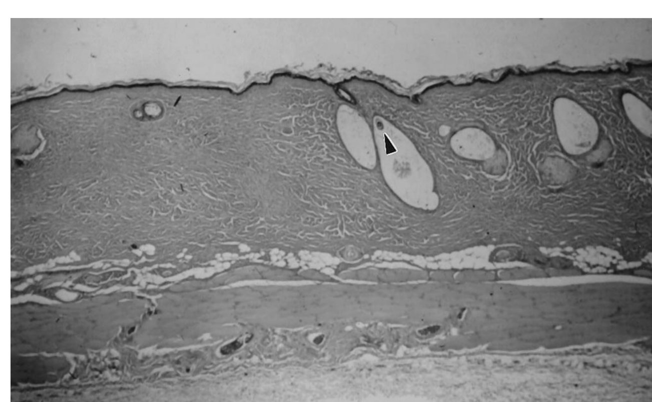

Fig. 8. *An auto/allo MCSG treated with DC at 3 months PG. Hematoxylin and eosin stain; original magnification, ×25. The cyst formed by the original allogeneic hair follicle has expanded and moved superficially. The hair shaft remnant contained brown pigment (*arrowhead*).*

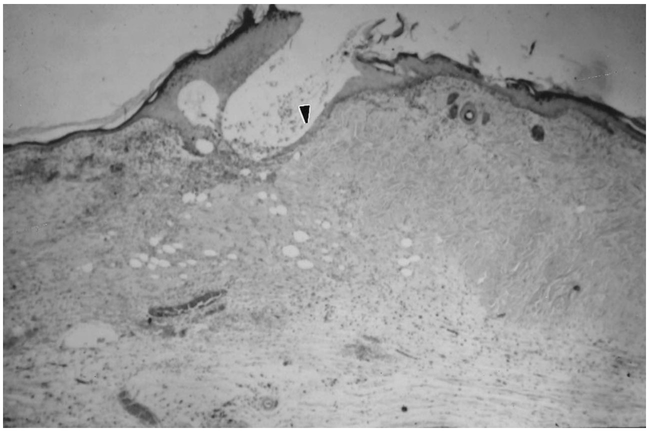

Fig. 9. *An auto/allo MCSG treated with DC at 21 days PG. Hematoxylin and eosin stain; original magnification, ×25. A cyst of rejected allogeneic hair follicle gradually moved superficially and eroded the autoepidermis. The debris, including the connective sheath, is then extruded through an opening in the epidermis of the MCSG. New epidermis is proliferating rapidly along the rough residual surface (*arrowhead*).*

Occasionally, during the early period of immune reaction of the allogeneic hair follicles in the auto/allo MCSGs, as shown in Figure 10 (10 days PG in the SNDC group), allogeneic hair follicle cells exhibited three types of dying cells, but no white cell infiltration, inside or around this hair follicle. These three types of dying cells are quite similar to the three types of the apoptotic cells.[29–31] This type of cell death during rejection may be a mechanism, which is limited to one of "local rejection" of the allogeneic hair follicles in the auto/allo MCSG.

4. Rejection of Allogeneic Cell Components Other than Hair Follicles: Excluding hair follicles, antigenic cells are of small number in the dermal allografts. Such cells are largely endothelial cells of the vessels, white blood cells, and cells of the excretory glands. Although rejection of these antigenic cells occurred, little influence on neighboring tissue was observed because of their minimal quantity in the dermal matrix. The vacuoles concealing the necrotic and eosinophilic cell debris because of cell rejection moved superficially and were expelled out of the MCSG after autolysis of a very small part of the autogeneic epidermis. New epidermal cells were migrating immediately beneath the debris. The inflammatory reaction was very mild in the SNDC group but obvious in the VG and SN groups. DC seems to accelerate eosinophilic debris excretion. In the SNDC group, collections of eosinophilic debris were evident within the first 2 weeks PG, but not thereafter. In both the VG and the SN groups, such vacuoles were evident at and beyond 3 months PG.

5. Dermis Generation: Auto/allo MCSG wounds were nearly completely covered by proliferated epithelial cells in 4 to 5 days with DC treatment, whereas in other auto/allo MCSG groups, epithelial coverage required more than 7 days. Early reepithelization of the open wound areas and allodermal surfaces provided not only an optimal environment for epithelial-mesenchymal interaction, but also the best protection from contamination. After reepithelization of the MCSG in these three groups (VG, SN, and SNDC), fibroblast infiltration under the new epidermis intensified. In SNDC wounds, no obvious inflammatory activity was observed. At 7 days PG, fibroblast infiltration and collagen deposition were evident beneath the epidermis, but no infiltration by other white cells occurred (Fig. 11). Mild white cell infiltration was noted in the SN group and was marked in the VG group. At 3 months PG, no obvious fibrotic tissue layer was found in the dermis of any experimental auto/allo MCSG wound; however, fine fibrotic tissue fibers homogeneously infiltrated the whole dermis slightly in the SN group and

Table 6 **Number of Rats with Cyst Formation in Auto/Allo MCSG Groups**

Group	Time PG (days)					
	4	7	12	14	60	90
VG[a]	0/5[b]	1/5	0/5	8/10	0/5	8/10
SN	0/5	2/5	4/5	2/10	4/5	8/10
SNDC	2/5	3/5	2/5	4/10	4/5	8/10

[a] Only epithelized areas of the wounds counted.
[b] Animals with cyst formation/no. of animals examined.

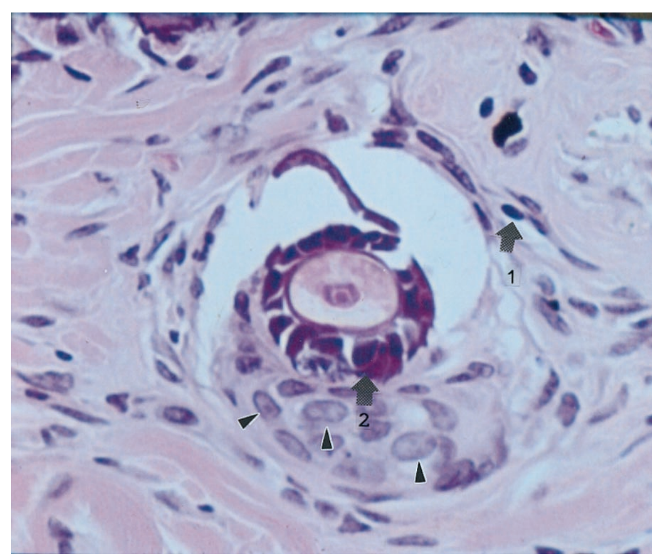

Fig. 10. *A cross-section of an allogeneic hair follicle in an auto/allo MCSG treated with DC at 10 days PG. Hematoxylin and eosin stain; original magnification, ×140. There are three types of dying cells evident in the hair follicle but no obvious white cell infiltration around the follicle. A single cell with a pyknotic nucleus and autophagic cytoplasm (vacuole) (*arrow 1*) similar in appearance to a pyknotic apoptotic cell is observed at the periphery of the hair papilla. A chain of dark-staining cells (original epithelial cell of external root sheath) surrounding a hair shaft can also be identified (*arrow 2*) like the apoptotic dark cells. In the adjacent light-staining cell with intact cell membranes (*arrowheads*), the microstructure of the nuclei and cytoplasm is broken down, as occurs in the shrunken cells undergoing apoptosis.[29–31]*

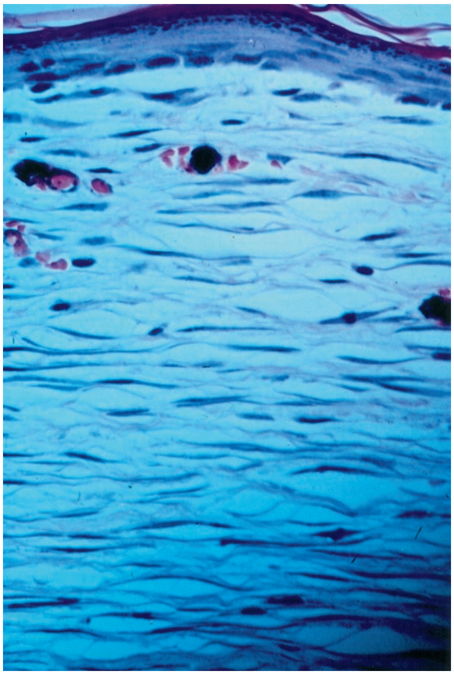

Fig. 11. *A high-power view of an original interstitial open wound area of an auto/allo MCSG treated with DC at 7 days PG. Masson trichrome stain; original magnification, ×120. There are no cells other than fibroblasts and collagen underneath the newly populated epidermis.*

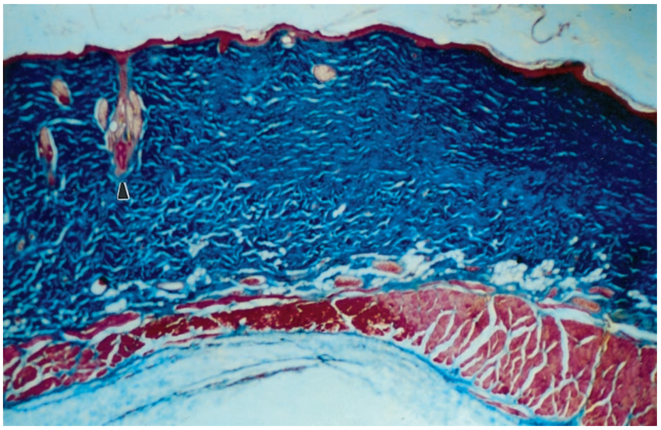

Fig. 12. *An auto/allo MCSG treated with DC at 3 months PG. Masson trichrome stain; original magnification, ×25. Arrangement of the dermal collagen is essentially normal. The right side of the figure depicts the double graft layer area (autograft/allodermal) containing essentially normal collagen dermis and a few hair follicles. The left side depicts the original allodermal and/or open excised wound area containing only dermal collagen, which is composed of a superficial longitudinal collagen fiber layer and a deep circular (referring to the body axis) collagen fiber layer with no hair follicles present.*

more obviously in the VG group. Such infiltration was not found in the SNDC group (Fig. 12). In the VG group, the healed wounds were characterized by heavy fibrotic infiltration. Consequently, the dermis of the VG groups was much thicker than that of the SN and SNDC groups (Table 7).

Table 7 **Dermal Thickness (mm) of Auto/Allo and Auto/Auto MCSG Groups at 3 Months Post Grafting**

Group	VG	SN	SNDC
Auto/allo[a]	1.290 ± 0.10	0.810 ± 0.04	1.004 ± 0.09
Auto/auto[b]	N/A	0.935 ± 0.05	1.087 ± 0.07

[a] In auto/allo MCSGs, the dermis of VG group, because of fibrotic tissue infiltration, is thicker than the SN and SNDC groups. Therefore, there is no need for statistical comparison among these three groups.

[b] SN vs. SNDC, $p = 0.074$. N/A, not applicable.

DISCUSSION

Insufficiency of available donor sites represents a formidable problem for extensive burn patients. In early 1960, a method developed to improve grafting in such patients involved the combination of two wound closure techniques: an intermingled (mosaic) skin graft combined of a sheet of the allograft and postage-stamp (0.3 × 0.3 cm) autografts; and repeated harvesting of skin from the scalp and soles of the feet to obtain the needed quantities of autograft.[1–4] When using this method, it was noted that, in some small areas, the epithelial cells of the autograft migrated into the gap between the allogeneic epidermal and dermal layers of the allograft, the so-called "sandwich" phenomenon.[4,5] No immunosuppressive agent was used. Such grafting was one of the earliest successful examples of composite skin grafts. Because of the need for repetitive operations, the time required (25 to 170 days) for complete wound healing, local persistence of granulation tissue, and frequent severe contraction, this method of wound closure was not readily accepted by most burn surgeons in the West.[1–5]

Currently, composite grafts are used for closure of extensive burn wounds, but they are limited by the need for multiple operative procedures, repeated grafting, and immunosuppressive therapy, as well as delayed healing and moderate to severe wound contraction. When cultured keratinocytes are used as the autograft layer in composite grafting, the day of grafting must typically be delayed for 3 to 4 weeks.[1–14,21,32–37] However, early escharectomy and grafting during the first week after injury are preferred by most surgeons for the treatment of extensive burns. In the present study, auto/allo MCSGs are shown to reduce or eliminate many of the problems associated with wound coverage in patients with massive burns. The composite skin grafts are applied in a single procedure to provide permanent wound coverage without need for either systemic or local pharmacologic immunosuppression. Additionally, the available autograft can be efficiently used in a 9:1 mesh. The wounds covered with MCSG are epithelized by 16 days PG (SN group) or 14 days PG (SNDC group).

Collagen is considered to be the major ground substance of the dermis. Type 1 collagen is the most ubiquitous of the dermal

collagens. Because the basic size, helical structure, and amino acid composition of many collagens within the same species have been highly conserved throughout evolution, collagen molecules are only mildly antigenic. Conversely, the epidermis and cellular components of the dermis, because of their surface membrane determinants, are active immunogens.[15,16,18,38,39,] Consequently, in allogeneic skin grafts of either full or partial thickness, the epidermis and cellular components of the dermis are acutely rejected, and dermal ground substances (matrix), i.e., chiefly the collagen, are only rejected after collagen has been denatured. In dermal allografts without immunosuppression, hair follicles and cell components, including vascular endothelium, are major targets for rejection. In the present study, we have generated data that are consistent with the hypothesis that the anatomy of the hair follicle restricts the rejection reaction of the allodermis. After the recipient produces antibodies to allogeneic tissues, the earliest rejection occurs in the endothelial cells of the capillaries and arterioles. Thrombosis then rapidly develops.[16,17] Rejection of allogeneic hair follicles appears to be continued within the surrounding connective sheaths without affecting neighboring dermal tissues. No obvious inflammation surrounding rejecting hair follicles is observed. Therefore, excluding hair follicles, the rejection activity of the small number of allogeneically potent dermal cells seems to be insufficient to influence the engraftment and healing of the allodermal layer. These findings suggest that dermal allografts, dermal xenografts, acellular dermal allografts, and even artificial collagen sheets (dermal analog) serving as components of an autograft/dermal composite skin graft may persist without immunosuppressive treatment.[33,40,41] Grafting method and graft dressing selection are other factors that may influence engraftment and enhance graft healing.

DC obviously accelerated restoration of the circulation in MCSGs. More importantly, DC seemed to enhance early recruitment of dermal vessels and dilated the vessels in both auto/allo and allo/allo MCSGs by 4 days PG before obvious rejection activity began. These changes in number and size of allodermal vessels are consistent with an increase in blood flow within the grafts. We also consider that when used in MCSGs, meshed dermal allografts (with many interstices) are superior to unmeshed dermal allografts, because they permit the meshed split-thickness grafts to establish contact a greater area of the open wound bed. That contact allows earlier revascularization and new vessel growth in both layers of the composite grafts. Meshed grafts also permit better wound drainage. If an allogeneic dermal graft can receive an early blood supply and be protected from contamination and/or infection without need for pharmacologic immunosuppressive therapy by using SN and SNDC as wound dressings, the allogeneic dermal collagen can be preserved and serve as the dermal substrate to support meshed autoepidermal graft healing and autogeneic epithelial cell migration from the meshed autograft. This experimental study also demonstrated that SN wound dressings with and without DC application could provide protection from wound contamination for at least 14 days, until complete epithelization and stratum corneum maturation had occurred. Such early epithelization and revascularization significantly accelerated wound closure and the remodeling of the healed wound.

A process of "local rejection" of allogeneic hair follicles appears to be important in the healing of the MCSG and protection of the allodermis from generalized rejection. As shown in Figure 9, the appearance of the three types of dying cells inside a rejecting allogeneic hair follicle of an MCSG is similar to that of the three types of apoptotic cells in the spontaneous involution of hair follicles during the catagen phase of the hair follicle life cycle.[29,30] Detailed studies are in progress to describe the temporal course of hair follicle apoptosis and its modification by low-amperage DC.

We can be certain that SN and SNDC have no immunosuppressive activity; however, in this experimental study, SN and SNDC, with their strong antimicrobial and advantageous wound healing effects, were definitely associated with persistent survival of the low antigenic allodermal tissue when applied in an auto/allo MCSG to an open wound.

CONCLUSION

Our results demonstrate the successful healing of a meshed auto/allo composite skin graft (MCSG) and apparent persistence of the allodermal tissue without pharmacologic immunosuppression. The use of widely expanded autoepidermal tissue permitted efficient use of available donor tissue in this murine model. We suggest that, after clinical trial, this MCSG technique can be applied in the clinical treatment of patients with extensive burns.

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